

**Title:**

**$\beta$ -glucan microparticles targeted to APN as mucosal antigen delivery system in oral vaccination of piglets against ETEC infections**

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**Abstract:** (Your abstract must use **Normal style** and must fit in this box. Your abstract should be no longer than 300 words.)

Enterotoxigenic *Escherichia coli* (ETEC) cause diarrhea in neonatal and newly weaned piglets, resulting in economic losses due to growth retardation, increased drug use and elevated mortality. Neonatal infections can be prevented by a passive lactogenic immunity obtained by vaccination of the sow, however, this passive protection disappears at the moment of weaning. To control post-weaning diarrhea, oral vaccination is a promising strategy, since vaccines delivered to the mucosal intestinal tract can elicit immune responses at the site of pathogen entry. Moreover, oral vaccination has many physiological and practical advantages, such as decreased costs and relative ease of administration without the risk of needle-stick injuries. Unfortunately, developing oral subunit vaccines has been challenging due to numerous potential obstacles, such as a hostile environment of the gastrointestinal tract, oral tolerance and the epithelial barrier. Therefore, antigen delivery systems in combination with selective targeting are an appealing approach to develop potent oral vaccines. The current study evaluated the capacity of aminopeptidase N (APN)-targeted  $\beta$ -glucan microparticles as oral delivery system. Antibodies against APN, an apical intestinal epithelial receptor, were conjugated to  $\beta$ -glucan microparticles via the biolinker protein G, which leads to a correct orientation of the antibodies. The resultant microparticles were analysed for their antigen encapsulation, adjuvanticity and interaction with enterocytes and dendritic cells (DCs). Targeting to APN is an efficacious way to increase the uptake of microparticles by enterocytes *in vitro* (IPEC-J2) and *ex vivo* (porcine intestinal explants). In addition, APN-targeted  $\beta$ -glucan microparticles are highly phagocytosed by DCs, which leads to the upregulation of DC activation markers CD25 and CD40 and to a strong pro-inflammatory cytokine response. Finally, conjugation of  $\beta$ -glucan microparticles with antibodies neither impeded antigen encapsulation nor adjuvanticity. Taken together, these data support the use of APN-targeted  $\beta$ -glucan microparticles for oral delivery of enteric pathogen antigens and motivate further *in vivo* research of these promising antigen carriers.